Spectroscopic Techniques for Pharmaceutical And Biopharmaceutical Industries

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UV-Vis Spectroscopy and its Applications.

Hello students welcome back to the lecture. We will continue with our discussion on UV visible spectroscopy and then we will got to look at the applications. In the last lecture I discussed about how to calculate energy of various electronic levels associated with conjugated molecule. Today I will focus on how to know the energy of different electronics orbitals of electronic materials.

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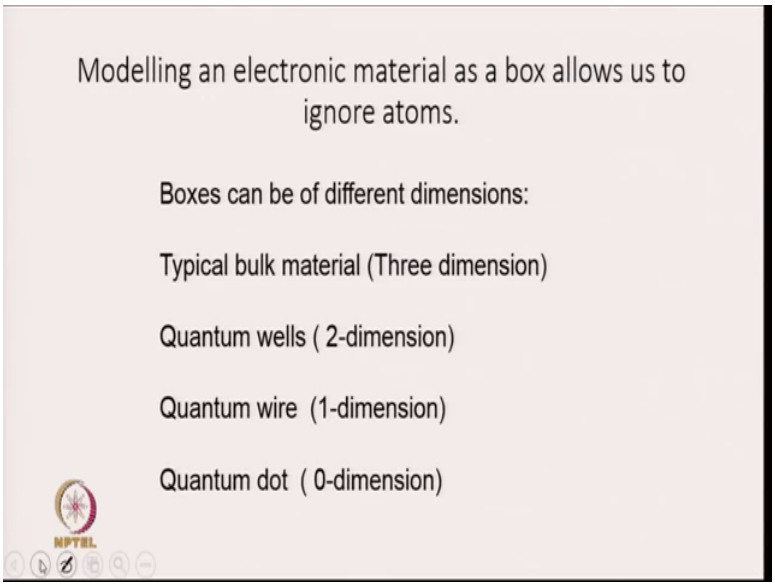

So modelling an electronic material as a box allows us to ignore atoms. So again we are going to apply the concept of particle in a box to model electronic materials. Here the box is can be of different dimensions quantum wells are known as 2 dimensional, I will explain what I mean by 2 dimensional in the next slide. Quantum wire is model as 1 dimensional material and quantum dot is considered to be 0 dimension.

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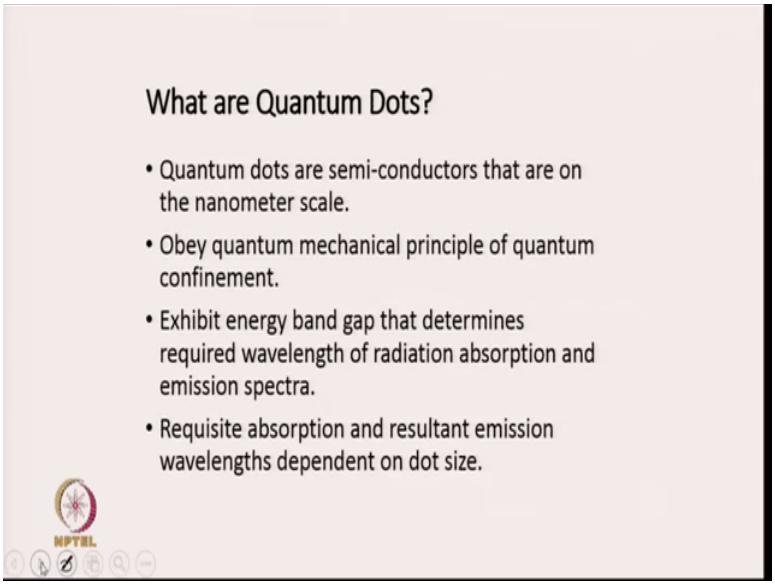

Now let us discuss about what is quantum dots? Quantum dots are semi-conductors that are on a nanometre scale and as the name suggest they obey quantum mechanical principle of quantum confinement. They show energy band gap that tells you about the wavelength of electronic transition either it is relate to absorption or emission spectra. What you will find out is that the absorption and resultant emission wavelength is dependent on the size of quantum dot, and that can be explained on the basis of modelling it as a particle in 1 or 2 dimension or 3 dimension.

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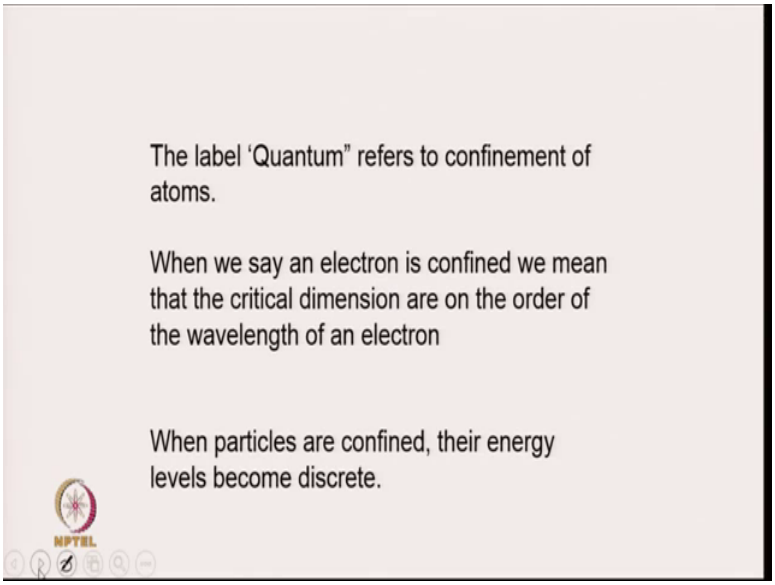

So what does the label quantum mean, Quantum label basically refers to confinements of atoms. So when I say that quantum dot is 0 dimension what I mean is that the quantum dot is

confined, the material is confined in all 3 dimensions why does this 0 comes? Because it is unconfined in any of the dimension you take X dimension Y dimension Z dimension it is unconfined in all three dimensions or in turns it means that it is confined in all three dimensions.

Now what we mean by confined? When we say an electronic is confined we mean that critical dimension are on the order of the wavelength of electron, and what is the result of it result is when particles are confined there energy levels become discreet that is what I have shown in the last lecture. So confinement results into energy level becoming discreet. So now let us again discuss what I mean by quantum well what I mean by quantum wire what I mean by quantum dot?

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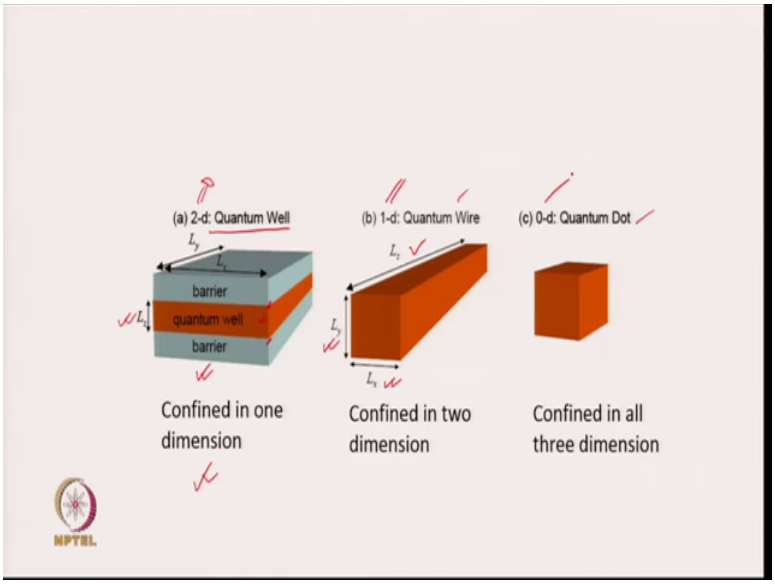

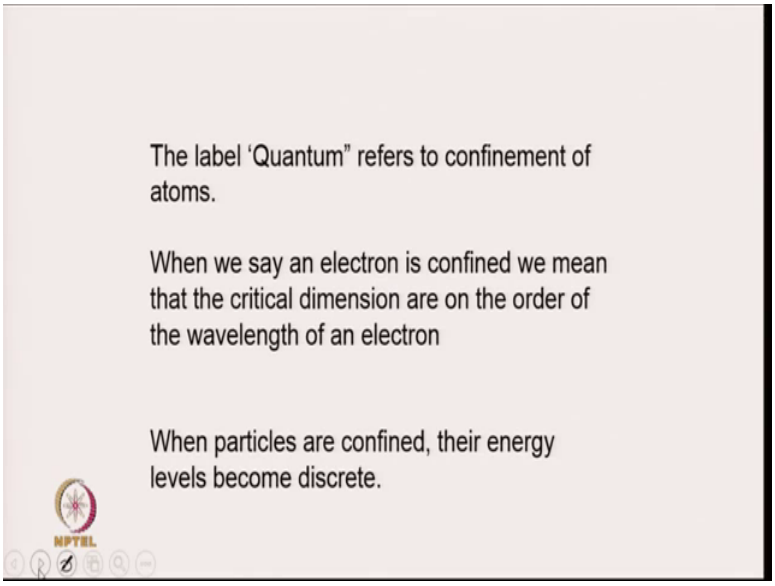

Now look at this figure this represents quantum well and it is called 2 dimensional material, 2 dimensional means it is unconfined in 2 dimension. So if we look at this picture, it is unconfined in X dimensions and it is unconfined in Y dimension where it is confined is Z dimension. So this is very small, this is your quantum well. So if you can look at here this size is very small, how much small that I already discussed with you that its critical dimension must be in the order of wavelength of an electron.

So that you need to keep in mind. So wavelength of an electron is the key, so in the Z direction it is of the order of wave length electron. So again 2 dimension means it is unconfined in X direction Y direction it is confined only in Z direction and that kind of material is known as quantum well. What is quantum wire? Now you see this is called 1 d material what does that mean, it is unconfined in 1 dimension.

What is that dimension that dimension is Z here. So you can see Lz is quite big, whereas the Ly and Lx is very small of the order of wave length of electron. So your quantum wire is confined in Y direction confined in X direction but unconfined in Z direction, and that is why it is known as 1 d material because it is unconfined in 1 dimension. Quantum dot in all three direction it is confined because length in all three dimension is of the order of wave length of an electron.

So this is known as 0 d because here is 0 is stands for the direction in which a material is unconfined since material is confined in all three directions. So there is no direction in which material is unconfined.

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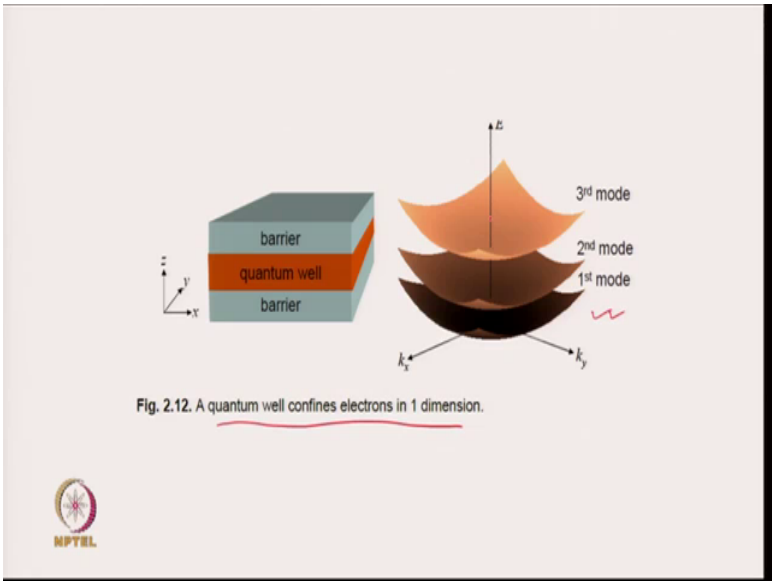

Fig. 2.12. A quantum well confines electrons in 1 dimension.

So now let us discussed about quantum well. As I told you that quantum well confines electron in 1 dimension in this case it is Z direction and so energy will take discreet value and this is your diagram. So this is your X axis this Y axis and this is Z axis and in the Z axis you have discreet energy levels.

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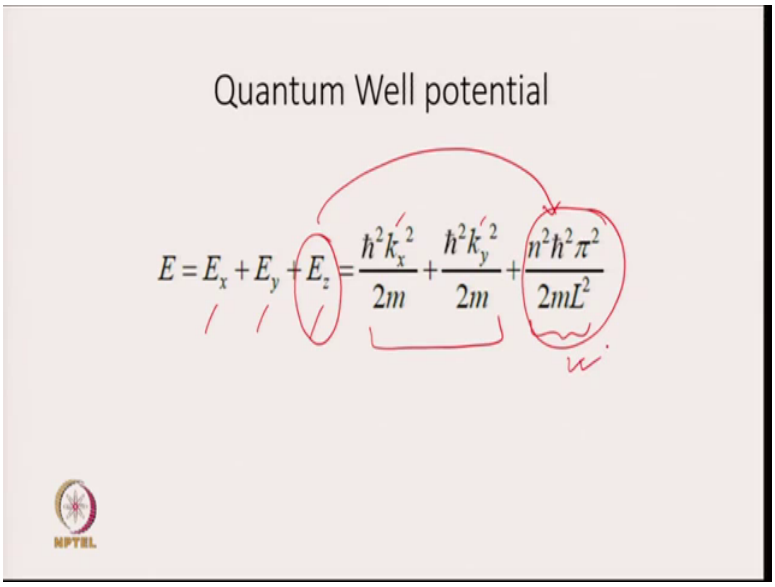

So how I can write the energy of quantum well energy of quantum well will be the sum of energy in X direction energy in Y direction and energy in Z direction and since in this direction in the Z direction the material is confined and so its energy will be given by n square h cross square pie square by 2mL square. That is what is the energy, or you can simply write n square h square by 8mL square.

This is when the, this is in the dimension in which your material confined for other two direction there is no limit on value of this key kx and ky it can take any value and so in this two directions the energy is going to be continues only in this direction energy can take discreet value.

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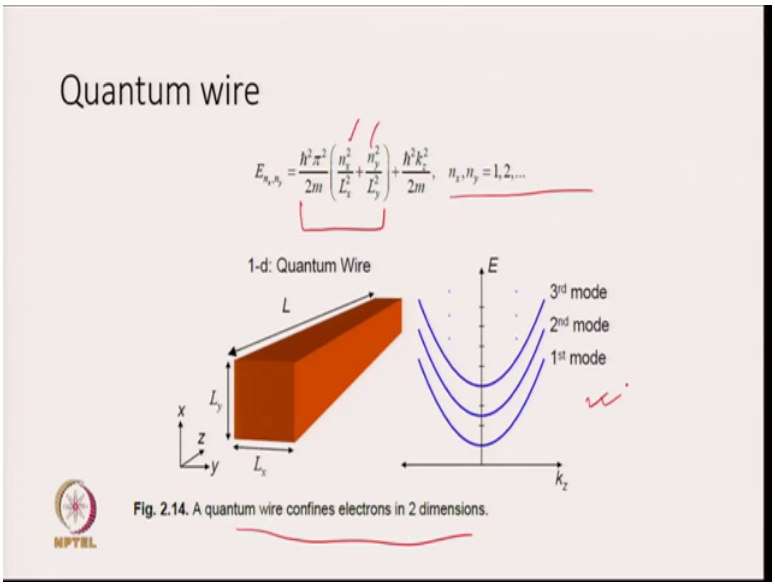

For quantum wire since it is confined in two direction it will depend on two quantum number nx and ny, nx and ny and nx and ny can only have value 1, 2, 3, 4. Hence your quantum wire is confines electron in 2 dimension and that is your energy level diagram.

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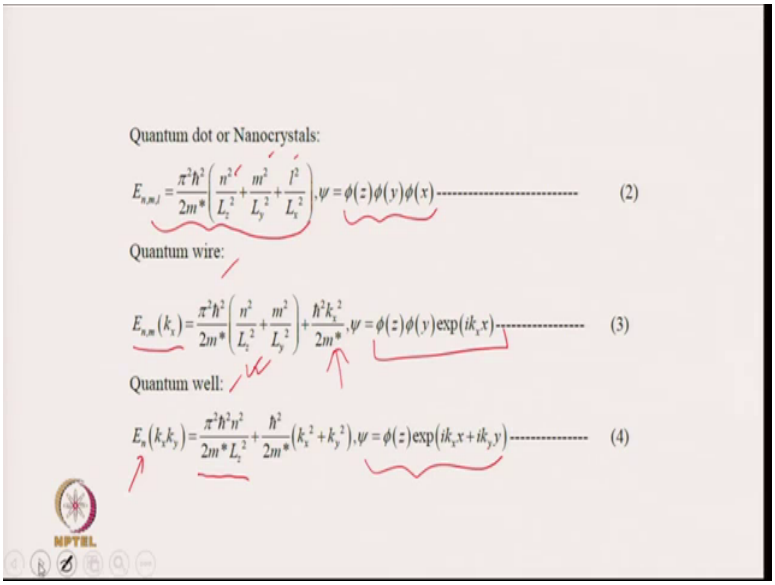

For quantum dot or Nanocrystal, since it is confined in all three directions so it will depend on 3 quantum number that is n, m, l and your equation will be this and the wave function can be written in terms of size equal to 5z 5y multiplied by 5x, 5z multiplied by 5y multiplied by 5x for quantum wire confined in 2 direction and so depend on 2 quantum number n and m and the energy will be given by this 2 terms and this is where it is not confined and your

wave function will be 5z multiplied by 5y and exponential ikx into x. In quantum well it is confined in only one direction that is Z direction so it will depend on n only energy will depend on n only and your wave function will be given by this equation. So this is the way you can model an electronic material.

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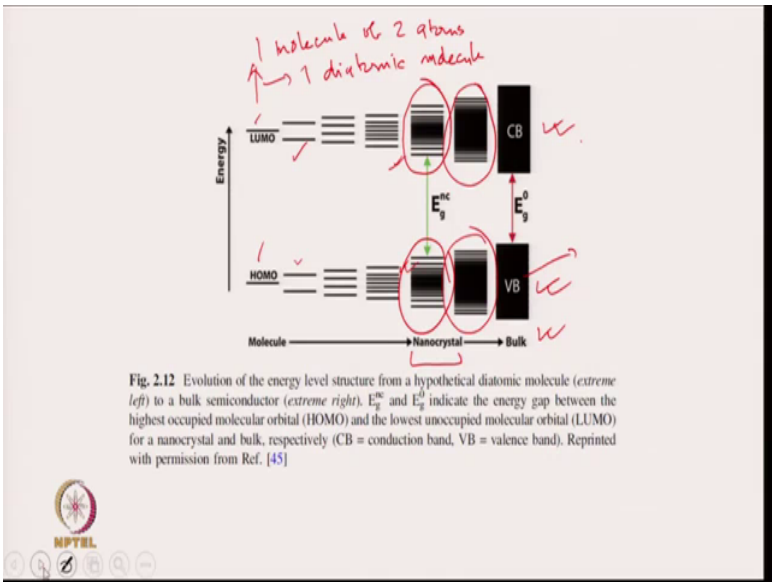

Fig. 2.12 Evolution of the energy level structure from a hypothetical diatomic molecule (*extreme left*) to a bulk semiconductor (*extreme right*). E_g^{nc} and E_g^0 indicate the energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) for a nanocrystal and bulk, respectively (CB = conduction band, VB = valence band). Reprinted with permission from Ref. [45]

Now let us think of how the evolution of energy levels takes place from a hypothetical diatomic molecule to a bulk semi-conductor. So what happens if suppose there are 2 atomic orbitals which combine to get 2 molecular orbital, for example HOMO and LUMO this is 1 molecule, 1 molecule of 2 atoms or you can say 1 diatomic molecule.

If you take 2 diatomic molecule you will get this kind of the structure if you increase the number of molecule then what will happen that here there will be kind of energies are overlapping with each other energy is overlapping with each other and that is when you have a nanocrystal, and you go to bulk you have a LUMO looks like this and HOMO looks like this. Now HOMO is known as balance valence band and denoted by VB and LUMO is known as conduction band and that is given by E naught. In nanocrystal this is the way LUMO looks like this is the HOMO looks like and the difference between this is given by Eg and c.

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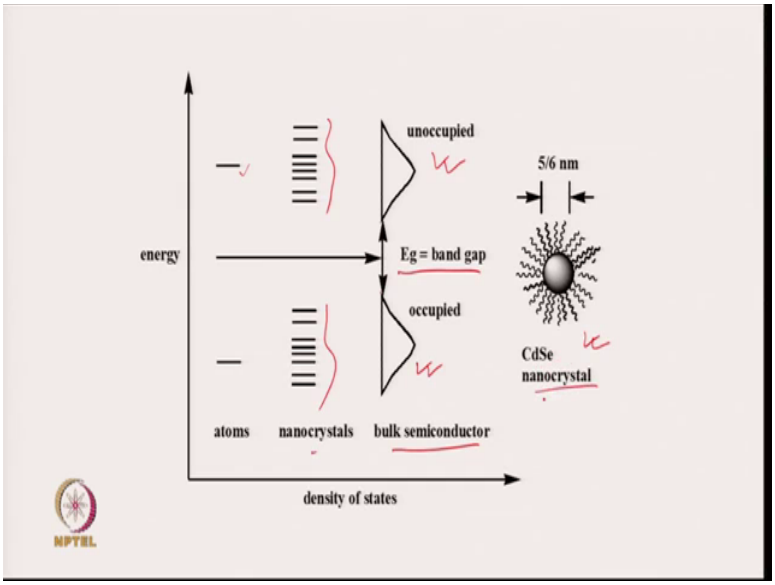

Now this is your energy profile for a nanocrystal or bulk semi-conductor so this is energy versus density of a state, so if density is low you have 2 energy levels and when you have a nanocrystal you have something like this and if you have a bulk semi-conductor you occupied level here and occupied level here and the difference between 2 level is known as band gap is known as band gap and band gap will depend on the size this is simple if you look at cadmium selenium nanocrystal, so it will look like this.

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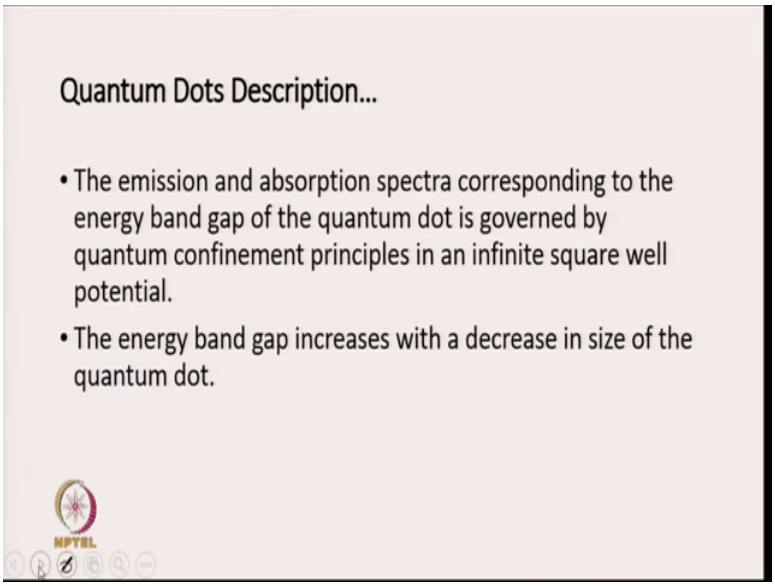

So if we look at quantum dots what we can see is the emission and absorption spectra corresponding to the energy band gap of the quantum dot is governed by quantum

confinement principle in an infinite square well potential, and the energy band gap increases with decrease in size of the quantum dot.

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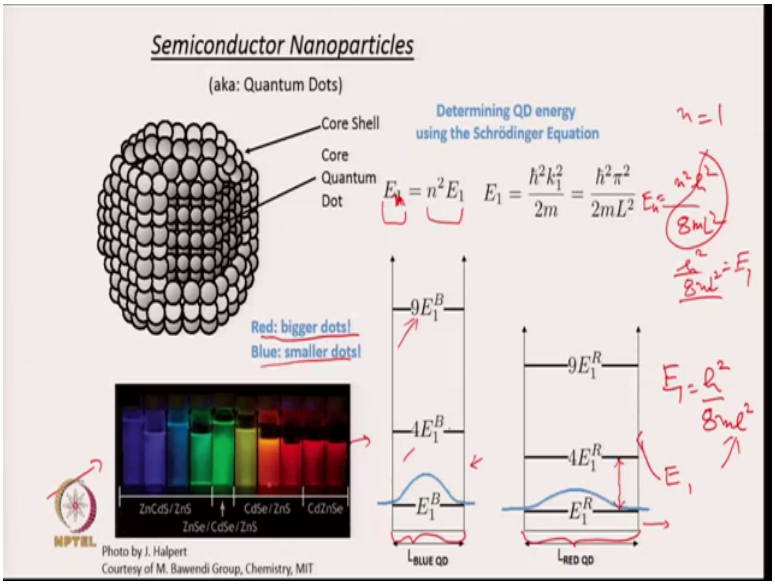

So let us look at here what is E1 if you remember this is n square h square by 8mL square unless write this as E1 go for n is equal to 1 or h square by 8mL square is equal to E1 and so En let us write En, is equal to n square so this is yours En. So I can write En is equal to n square in to E1 since E1 is equal to h square by 8mL square. So if n is equal to 1 so it is simply E1 if n is equal to 2 you have a 4 E1 and n is equal to 3 and you have a 9 E1.

Now let us look at if I increase the size, size of your quantum dot what will happen. So when I increase the size you see here I am going from this size to this size so certainly this size is larger. So in that case if you remember E1 is equal to h square by 8mL square and since L is high L is large and so E1 in this case is going to be small compare to E1 in this case and now you see E1 R is going to be smaller than this

And this 4 E1 is going to be much smaller than for 4 E1 B and so gap between two energy levels will be small gap between energy levels will be small when you have bigger quantum dot bigger quantum dot what does that means is that if delta e is small it means lambda will be large and so if you are going from smaller quantum dot to a bigger quantum dot what you will get is something like this colour will change from blue to red.

Red means bigger dots and blue means smaller dots. So this is your smaller dots this is for smaller and this is for bigger as the size of quantum dots increases you move from smaller

lambda to higher lambda hence you are going from blue to red, blue to red. So this is the way you can explain the colour of quantum dots.

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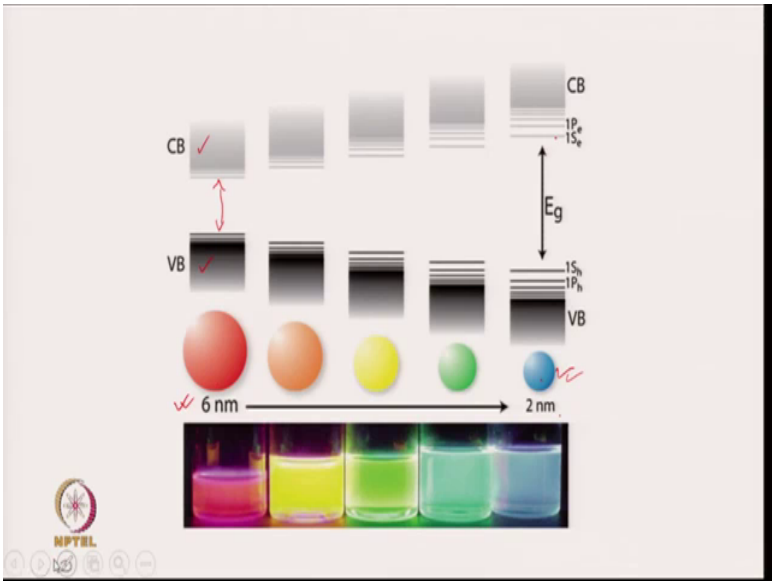

And again that you can show by the energy profile so here is conduction band here is the valance band. When this is small then you see the reds colour when this is large you see blue colour and red you get when the size is big blue you get when size is small. So if quantum dot size is big then energy gap is small and lambda is high when the quantum dot size is small then energy gap is larger and lambda is smaller, lambda is smaller.

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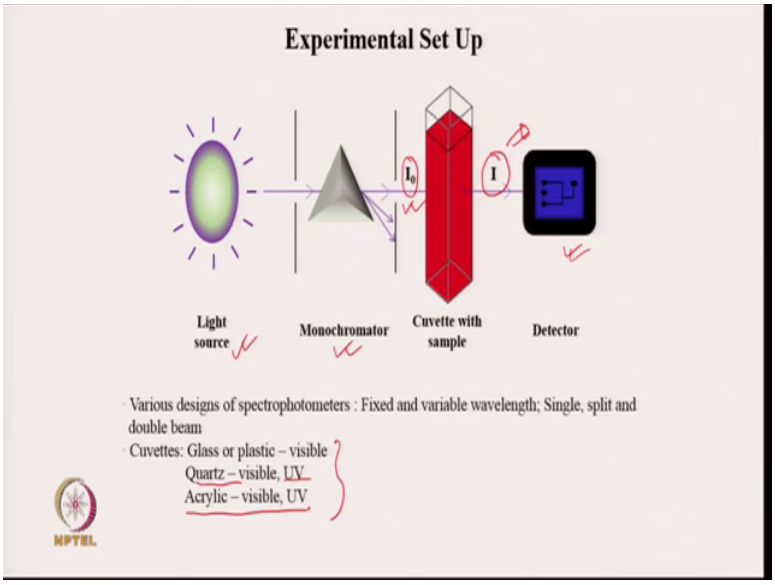

So this is about your, this is about the way we explain we explain the colour of nanocrystals. Now we will go and look at the experimental set up. So in the experimental set up there are 2

very important component one is your light source and another is monochromator. So first light source will go through monochromator and then it passes through sample and sample absorbs some radiation rest of the radiation is detected by a detector.

So what is happening that light crosses through sample when light crosses the sample the intensity of the light will decrease from I naught to I and this is because a part of the light will be absorbed by the sample in the cubic and what we do is we measure this intensity we already know I naught based on that we can calculate fiscal parameter known as absorbance there can be various type of spectrophotometers fixed and variable wavelength single split and double beam.

Cuvettes are this so you can use glass or plastic cuvettes for visible reason but not for UV reason. Quartz is needed if you want to measure in UV you can sometime use acrylic also if you want to measure in UV reason. Now one of the important part is monochromator what monochromator does?

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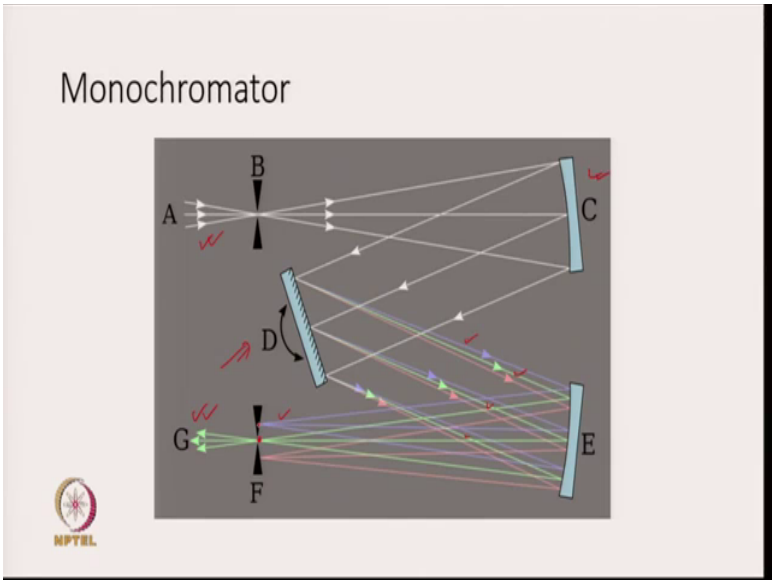

So what monochromator does is you see here is your light, light is first reflected by this mirror and then this goes to this D and what it does it basically split this light into various wave length you again keep a mirror and what is it doing just look at it what is it doing is that all, for example this colour let us take green colour. So green, green and this is green all three green colour is now comes and meets at one point and this is the way you are able to select a particular wavelength, particular wavelength all this pink one will come at one point all this what about this colour is violet it comes at one point.

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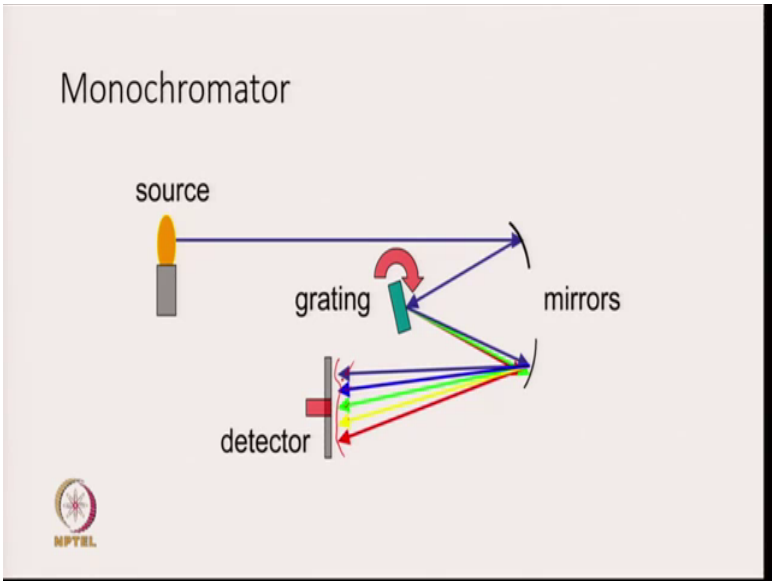

So what is being done first light from the source is reflected by mirror and it is split by a grating and then again it falls on a mirror what it does is it is split the light into different wavelengths light. So and now here is a detector now what you can do is depending on the wave length at which you want to look at you want to look at the absorbance you can just move detector .So sometime first you can look at the green one then you can look at blue one and then so. At every lambda you can look at what is the absorbance at every lambda you can look at what is the absorbance and this way your spectrophotometer works.

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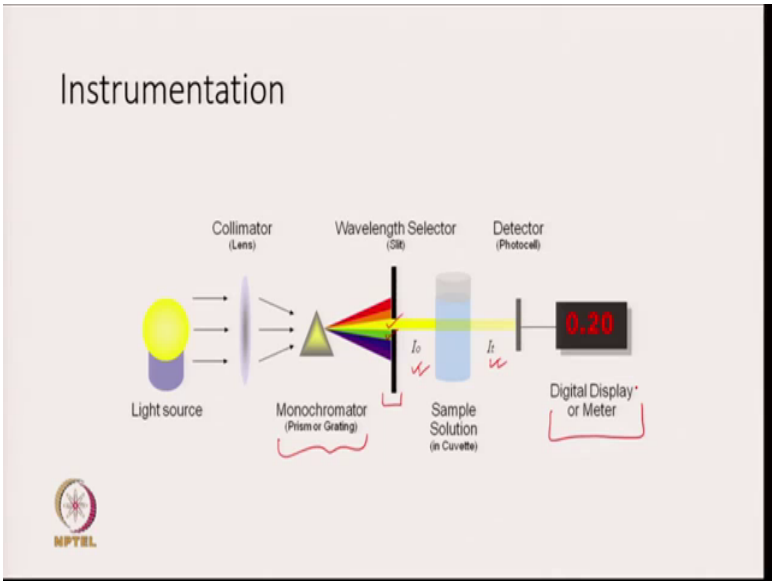

So light source lens then it passes through monochromator which is either in form prism or a grating what it does as I told you that it will split light into various colours. You select one of

them using slit which is basically x like wavelength selector so you can move it. So say for example in this figure yellow light has been selected but if you move it down then it will select the green light and so on. You pass through sample solution and what you get is intensity after the absorption of it in sample. Now this two things we know, and based on this two things digital display will tell you about absorbance. Now we will discuss about what is absorbance.

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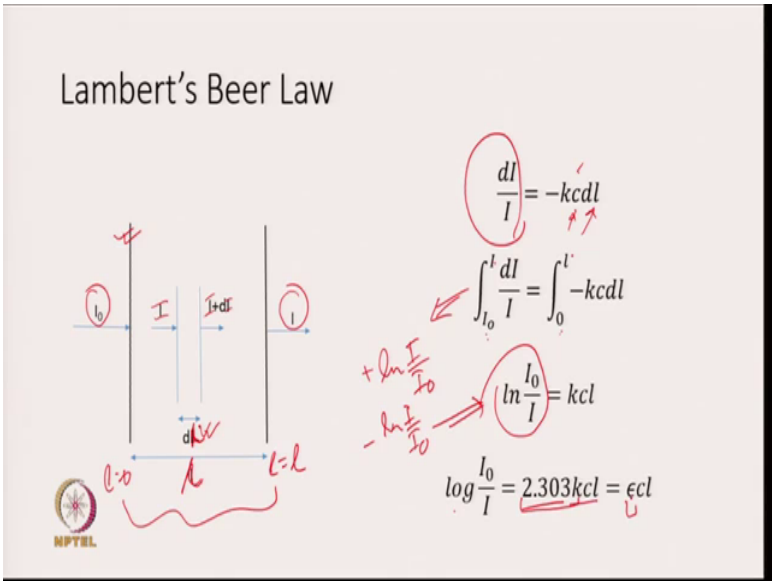

So the numerical expression is given by Lambert's beer law. So now look at, this is you suppose cuvette this is your cuvette and then cuvette containing sample and then what happen that you are sending light which intensity is I naught and when this light passes through sample it gets you intensity I rest of the intensity is rest of the intensity is absorb by the sample.

So let us think about small very infinite decimal volume in this cuvette. So let us think about that if I here I intensity and that changes to I plus dI and it passes through this sample this small volume and the length of this is dl, so this is dl do not get confuse this is d small l, d small l. And this total length is l this total length is l. So your decrease in intensity is of first order with respect to concentration.

So dI by dt will be proportional to concentration and proportional to this small length which is dl small length and dl minus sign comes because I decreases when it passes the sample. Now let us indicate this between I naught to I and that is when length is 0 to length is equal to l, remember here length is 0 and length is l and here intensity is I naught and here intensity is I. So you are integrating intensity between I naught to I and length between 0 to l.

What you will get is log ln I naught by I, I naught by I. So this will be I have taken this minus sign here and so this will be ln I naught by I. If you integrate this, this will become minus ln or plus ln I by I naught for since have taken negative sign here so I can write minus ln I by I naught and that is nothing but equal to ln I naught by I is equal to kcl. If you take logarithm at the base 10 then log I naught by I is equal to 2 point 303 kcl and this 2 point 303 into k is known as epsilon value. So epsilon into c into l.

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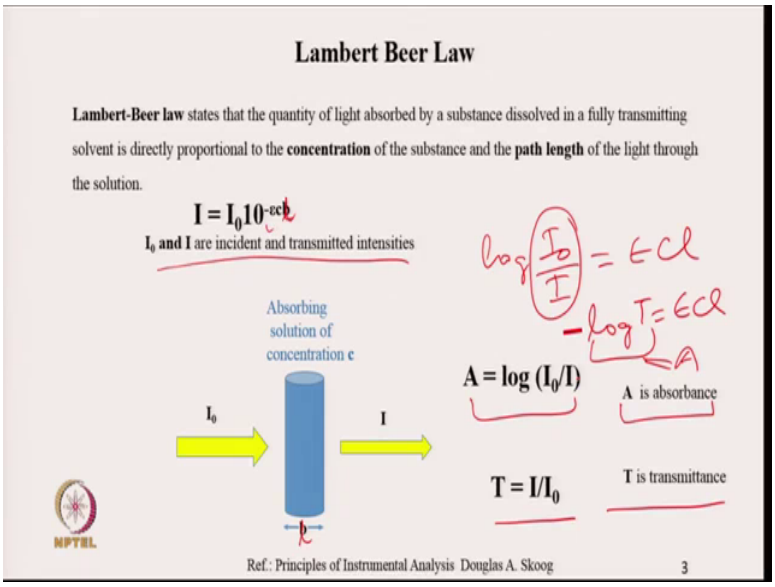

So what Lambert's law tells you that quantity of light absorb by a substance dissolved in a fully in transmitting solvent, is directly proportional to concentration of the substance and the path length of the light passing through the solution. So I is equal to I naught 10 power minus epsilon cl this is length where I naught and I are the incident and transmitted intensity this length is l.

Now this we got what log I naught by I is equal to epsilon cl this I0 by I is known as transmittance. So minus log T is equal to epsilon cl where T is transmittance look here and this minus log T is called absorbance. So your A is you're absorbance and that is given by log I naught by I. So if you pass light intensity of the light decreases when it passes through the solid state. But now what we are measuring is absorbance which is log I naught by I and since I naught is always going to be greater then I and so A is going to be going to have a positive value A is always going to be positive value. If A is large it means I is small I naught by I is large and since I naught is a constant and so I is going to be small, I is going to be small then absorbance is going to be high.

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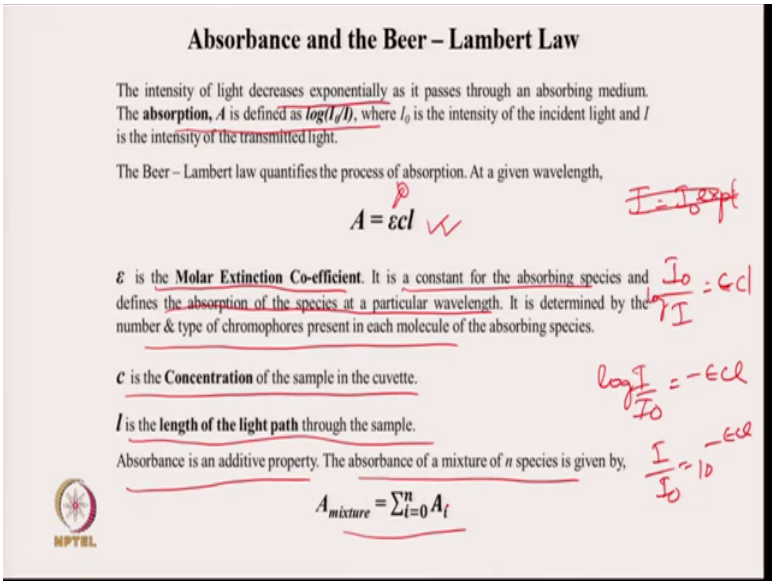

Intensity of light decreases exponentially as it passes through an absorbing medium. So we have already written that I is equal to I naught exponential minus if remember I naught by I log is equal to epsilon cl and so let us just cancel this out. So log I by I naught is equal to minus epsilon here or I by I naught is equal to 10 power minus epsilon that is what we wrote earlier. So you can see that I decreases exponentially this is your beer lambert's law, what is that A is equal to epsilon cl.

This Epsilon is known as which is a constant molar extinction co-efficient. It is constant for the absorbing species at particular wave length and basically it defines absorption of a species at a particular wavelength. It is a constant for a given species at particular wavelength when if our species is seen absorbance or epsilon can differ at different lambda value but for a given compound at given wavelength epsilon is constant.

And the value of epsilon will depend on the number and type chromophores presenting each molecule of absorbing species and we have already talked about on what factor intensity and spectra depends on basically epsilon depends on all those factors. C is the concentration of the sample in the cuvette l is the length of the light path through the sample and one of the most important property is absorption is an additive property and this is very important because you will later see that this can be used to know the thermodynamic and kinetic parameter of a reaction. So absorbance of mixture of n species is given by I is equal to 0 to n AI.

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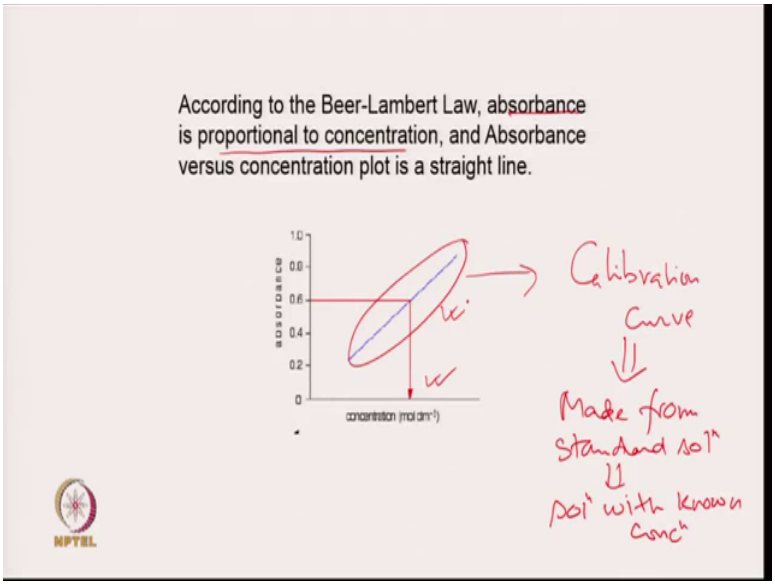

One of the most important application of beer lambert's law is to get the concentration and what the beer lambert's law tells you that absorbance is proportional to the concentration and so the plot between absorbance versus concentration must be a straight line. So it is here is a straight line and if you know what is the absorbance of the sample you can tell what is the concentration and this line is called calibration curve and that is made from standard solution.

By standard solution I mean solution with known concentration. So first you may calibration curve and if you know what is the absorbance at a particular wavelength absorbance at a particular wavelength in an unknown sample absorption of compound at a particular wavelength in an unknown sample you can know the concentration by using calibration curve for that compound calibration curve for that compound.

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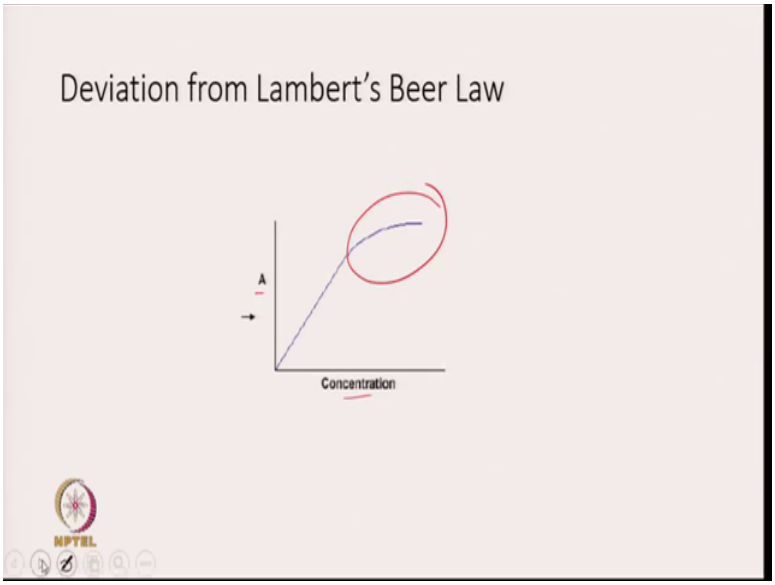

But it is not always true that absorbance versus concentration. So it is your linear curve. What happens is at high concentration you can see the deviation due to.

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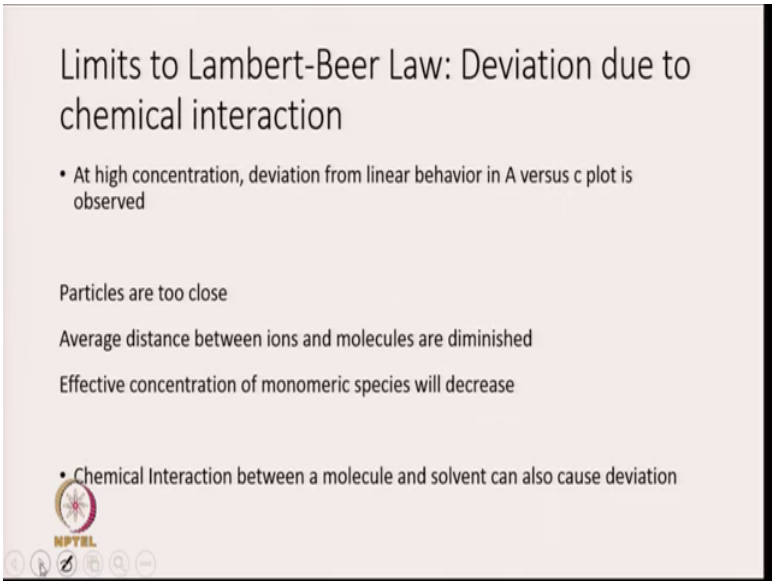

What is limits to beer lambert's law or lambert beer law? The deviation could be due to chemical interaction. At high concentration deviation takes place from linear behaviour or linear deviation from linear behaviour in A versus concentration plot is object. What can be reason, reason can be that particles are too close and they basically effects each other charges.

The average distance between ions and molecule are diminished and so what will do is there is more chances of interaction between themselves, between themselves and that may lead to association and so basically effective concentration of monomeric species will decrease. The

deviation can also happen because a molecule may interact with solvent and that can give you deviation.

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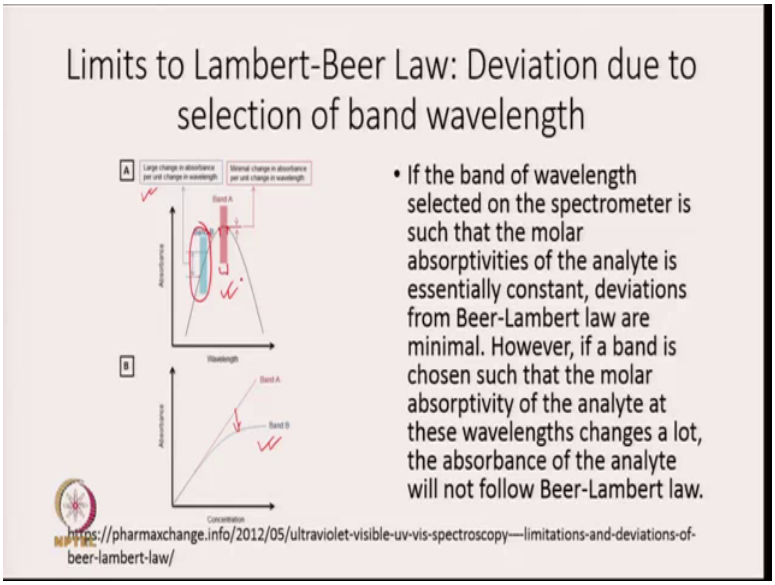

The deviation can also be due to selection of band wavelength, band wavelength. So suppose I try to absorb within this band here you can see there is minimal change in absorbance per unit in wavelength. So if you go from this point to this point change in absorbance is not very high but if you suppose I take here change in the wavelength will lead to a large increase in absorbance.

So large change in absorbance per unit change in wavelength. So what will happen if I try to plot absorbance versus concentration if I measure a concentration in this wavelength range I will get this kind of curve but if I try to measure in this band then I will get a nonlinear curve and it will deviate from straight line there will be deviation from straight line. So if the band of wavelength selected on the spectrophotometer is such that molar absorptivity of the, an light is essentially constant deviation from beer lambert law wavelength.

And that is what happens when we are selecting band wave. However, if a band is chosen such that molar absorptivity of the light at this wave length changes a lot then the absorbance of the length will not follow beer lambert's law.

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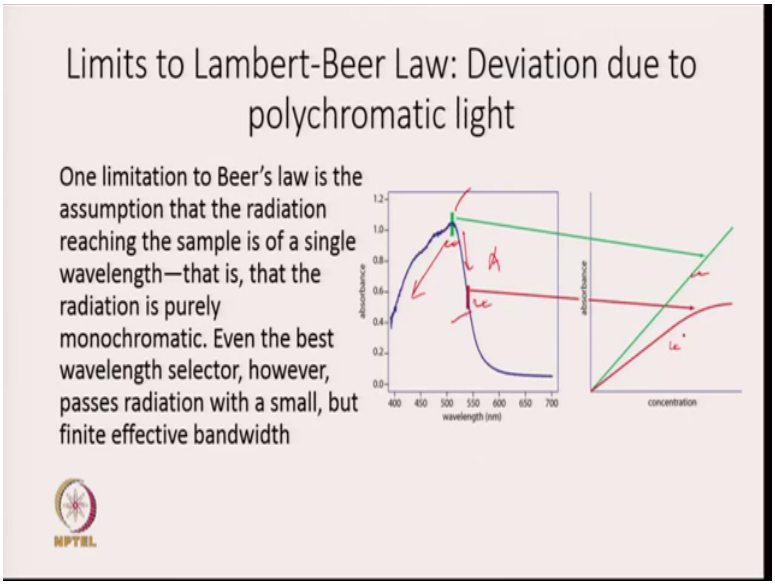

There is another limitation that is due to use polychromatic light. So absorbance of a compound depends on wavelength of the light which we are using to get the concentration or get the absorbance. For example, if suppose this is spectra of a compound and if I chose a wavelength here then your absorbance is going to high if I chose a wavelength here then absorbance is going to be low.

Suppose I have polychromatic light it means a mixture of a certain range of wavelength. What will happen you can see here there is a decrease there is a decrease and so what will happen is that your absorbance will be sum of, sum of absorbance at this wave length and at this wavelength or within this range and so the absorbance will not be high it will so low. So let us look at here if I measure at this wavelength I will get this curve if I measure at this wavelength I will get this curve and your absorbance is a mixture of this two and so there will be deviation towards the smaller absorbance value.

What does it means is the assumption that the radiation reaching the sample is of single wavelength is generally not true the radiation are generally mixture of wavelengths and thus they are polychromatic they are not monochromatic and because of that what are observing is smaller absorbance and so deviation from beer lambert's law takes place even if you take very good wavelength selector you may not be able to get a single or a radiation of single wavelength is always going to is always going to get mixtures of few wavelength, mixture of few wavelength and because of that the absorbance versus concentration curve will not exactly follow lambert, beer lambert's law.

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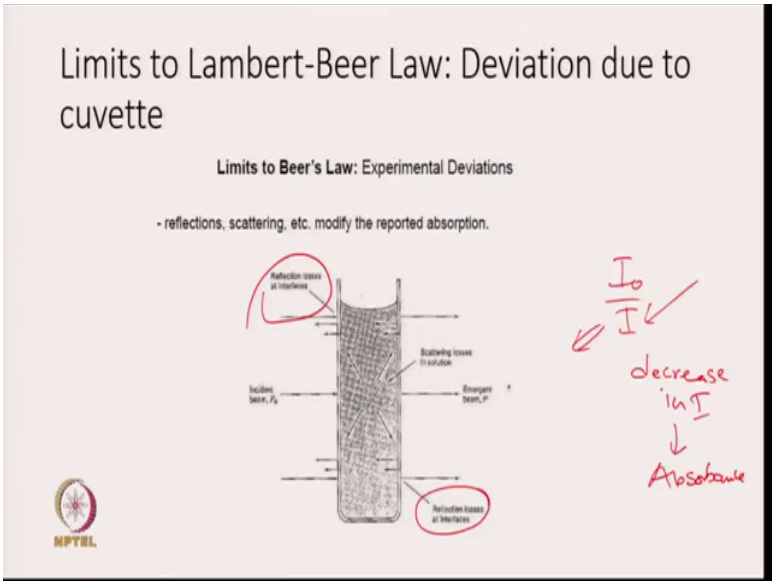

There are other limitations and that may be due to the kind of cuvette you were using and you can look at that there can be, so let us think about this what we are measuring we are measuring the intensity of the light when it passes through the sample and we are looking at the ratio between I naught versus I and what we are assuming that the decrease in I, decrease in I is only due to absorbance.

Absorbance applied by the sample but the intensity may also decrease due to reflection losses at interface. So you see if suppose your material of the cuvette reflects the light then the light is not passing through the sample. So apart of intensity is lost due to reflection there can be reflection here, so interface again at the interface and so there is your decrease in intensity this decrease is not due to absorption, this decrease is due to reflection.

And what can also happen is that there are suppose dust particle in our solution our sample they will scatter the light there are bigger particle in the sample they will scatter the light. So light can be lost by other process than absorption other process other than absorption in that case I naught by I is going to be much higher in comparison to when your decreasing intensity is only due to absorption. So you may see higher absorbance you may see higher absorbance in comparison to a perfectly normal absorption measurement perfectly normal absorption measurement.

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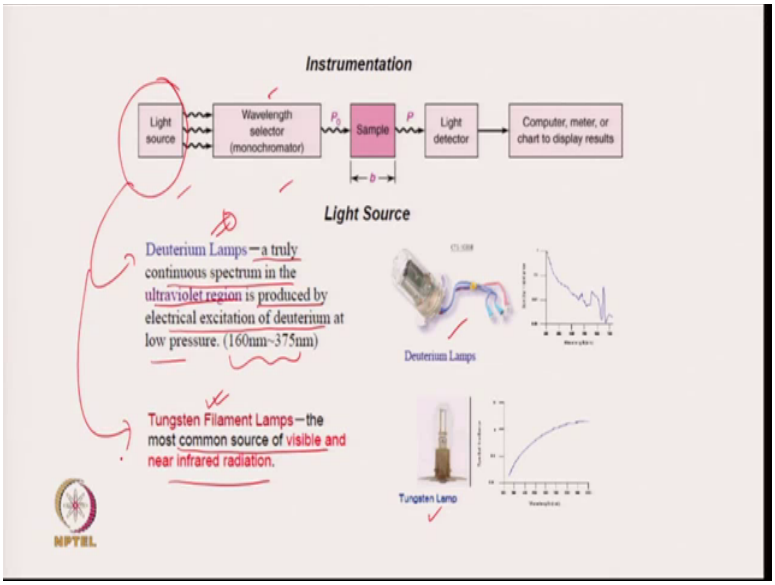

So this is about your wave length selector now I will also talk about light source there are two different kind of light source you can use and one is deuterium lamps this is basically gives you a truly continues spectrum in the ultraviolet region and is produced by it is produced this spectrum is produced by electrical excitation of deuterium at low pressure and it basically gives light in the region 160 nanometre to 375 nanometre.

So this is generally used for ultraviolet region so this is Tungsten filament lamp is most common source of visible and near infrared radiation. This is your deuterium lamp and this is your tungsten lamp this already we have discussed I have already discussed wavelength selector. So here is the light source, so light source can either be deuterium lamp or tungsten filament lamp.

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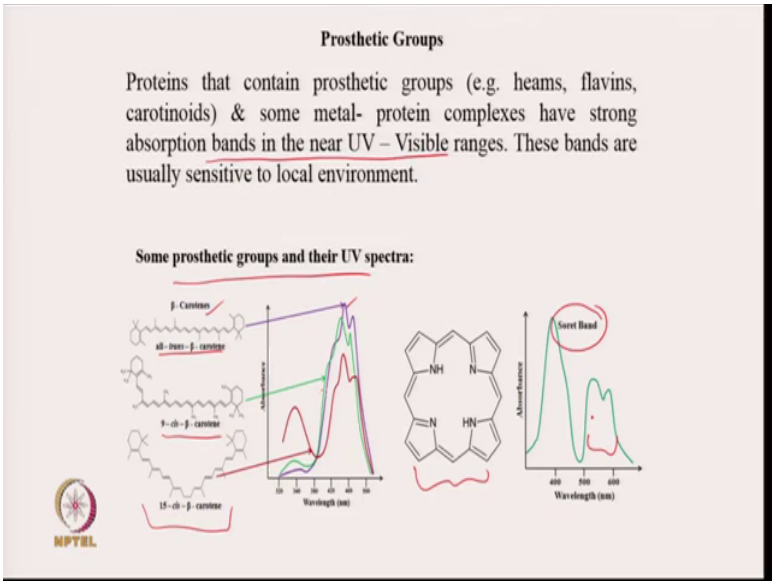

Now I will move towards the application of absorption spectroscopy. So what we can look at is if you want to analyse a molecule here it is a protein molecule and we have to look at we have to look at what are the prosthetic group in that molecule they may have an absorption band in near UV visible regions and this bands are usually sensitive to local environment, and so we can look at when the effect of environment by looking at the spectra of prosthetic groups.

So here are some prosthetic group and the UV spectra. For example beta carotene this is the spectra this is all trans beta carotene if 9 6 beta carotene you take it will have different spectra and 6 beta carotene you take it will have different spectra and this is your (())(46:18) group it has a well-known solid band and there are some other pits other bands. So each chromophore has each chromophore has their specific UV-visible spectra and based on that we can know you can qualitatively determine the presence of a chromophore. We can also quantitatively determine the amount of chromophore in that solution the amount of chromophore in that solution.

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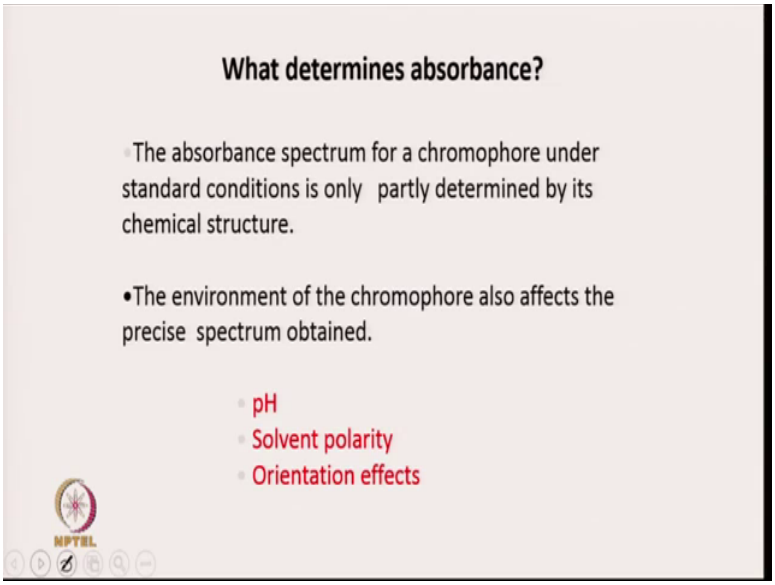

So what determines the absorbance spectrum for a chromophore under standard condition is only partially determine by its chemical structure. So it is not always true that only chemical structure determines the UV visible spectra.

Environment of the chromophore can also effect the precise spectrum obtained and what are the different environment you can think of. So pH of the solution will determine how the spectra of a particular chromophore look like solvent polarity the solvent which you are using to make the solution a solvent in which your particular compound is a particular compound is there that is also going to determine the precise spectrum of that molecule and similarly or intension effect can also determine. So UV visible spectroscopy is quite often used for qualitative analysis it is not only use to for structure determination of a organic compound. It can also be use for determination of impurities.

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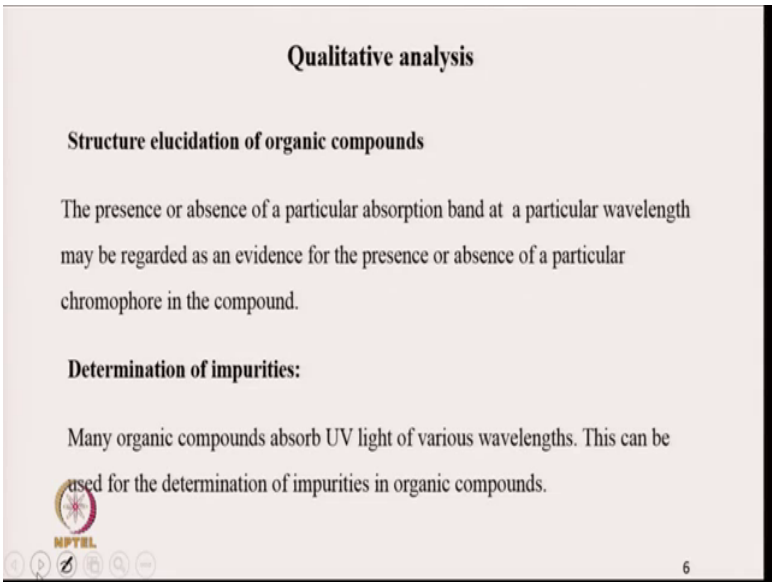

The presence or absence of a particular absorption band at a particular wavelength may be regarded as an evidence for the presence or absence of particular chromophore in the compound. So I told you that each chromophore has different absorption spectrum some may absorb at suppose 500 nanometre some may absorb at 550 nanometre some may absorb at 350 nanometre.

So based on that you can by just looking at the UV-visible spectrum you can tell which kind of chromophore is present in the compound. So suppose if you are carrying out a reaction and you got a particular organic compound first thing you can do is go and take your visible spectra of that compound and may be you can get some idea about what is the chromophore present in that compound.

Not only that, it can also be used for the determination impurities. So whenever you are making some compound there are chances of impurities being present in that sample. So you can tell about impurities by looking at UV visible spectra because the impurities which are mainly organic compound will absorb UV light or various wavelength and that wavelength will be may be different wavelength at which your product or reactant absorb and based on that you can tell about whether there is presence of impurity or not.

So UV visible spectroscopy is quite often used for the determination of impurities in organic compound. We can also go for quantitative analysis I have already told you that absorbance is

proportional to concentration. So is we measure absorbance at a particular wavelength we would be able to tell about the concentration of the compound.

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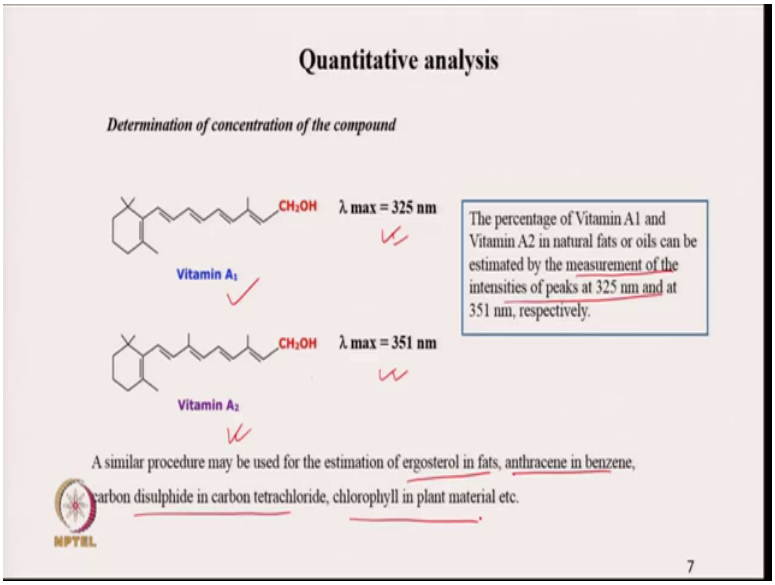

For example, vitamin A1, vitaminA1 absorbs at lambda max is equal to 325 nanometre lambda max is equal to 325 nanometre whereas vitamin A2 absorb at max is equal to 351 nanometre. So the presence of vitamin A and vitamin A2 in natural fats or oil can be estimated by measurement of intensity of peaks at 325 nanometre and 351 nanometre respectively. So just by looking at absorption spectra and looking at the intensity of the peak a 325 nanometre which belongs to vitamin A1 or 351 nanometre which belongs to vitamin A2. You can know the concentration vitamin A1 and vitamin A2 in natural fats.

Similar kind of procedure has been used for estimation of ergosterol in fats anthracene in benzene carbon disulphide in carbon tetrachloride and chlorophyll in plant material. So I was started to discuss application of UV visible spectroscopy the lot of applications and since now time is over I will stop here and I will talk about more application in the next lecture thank you thank you very much for listening.